

1. A method of sequencing a nucleic acid molecule comprising steps of:

providing two separate, adjacent solutions of a medium and an interface between the two pools, the interface having a channel so dimensioned as to allow sequential nucleotide-by-nucleotide passage from one pool to the other pool of only one nucleic acid molecule at a time;

providing a nucleic acid molecule with at least one repeat of a nucleotide sequence to be determined, wherein the nucleic acid molecule is enzymatically synthesized using a circular template, and wherein the nucleic acid molecule contains modified nucleotides that reduce secondary structure in the nucleic acid molecule;

placing the nucleic acid molecule in one of the two pools; and

taking measurements as each of the nucleotides of the nucleic acid molecule passes through the channel so as to determine the sequence of the nucleic acid molecule.

2. The method of claim 1, wherein the nucleic acid is single-stranded.

3. The method of claim 2, wherein the nucleic acid is single-stranded DNA.

4. The method of claim 2, wherein the nucleic acid is single-stranded RNA.

5. The method of claim 1, wherein the nucleic acid is an unstructured nucleic acid.

6. The method of claim 1, wherein the circular template is single-stranded.
7. The method of claim 1, wherein the circular template is double-stranded.
- 5 8. The method of claim 1, wherein the medium is electrically conductive.
9. The method of claim 8, wherein the medium is an aqueous solution.
- 10 10. The method of claim 9, further comprising applying a voltage across the interface.
11. The method of claim 10, wherein ionic flow between the two pools is measured.
- 15 12. The method of claim 11, wherein the duration of ionic flow blockage is measured.
13. The method of claim 11, wherein the amplitude of ionic flow blockage is measured.
- 20 14. The method of claim 8, further comprising applying a voltage across the interface.

15. The method of claim 14, wherein ionic flow between the two pools is measured.
16. The method of claim 15, wherein the duration of ionic flow blockage is
5 measured.
17. The method of claim 15, wherein the amplitude of ionic flow blockage is measured.
- 10 18. The method of claim 1, wherein the nucleic acid polymer interacts with an inner surface of the channel.
19. The method of claim 18, wherein the medium is electrically conductive.
- 15 20. The method of claim 19, wherein the medium is an aqueous solution.
21. The method of claim 20, further comprising applying a voltage across the interface.
- 20 22. The method of claim 21, wherein ionic flow between the two pools is measured.

23. The method of claim 22, further comprising applying a voltage across the interface.

24. The method of claim 23, wherein ionic flow between the two pools is
5 measured.

25. The method of claim 24, wherein the duration of ionic flow blockage is measured.

10 26. The method of claim 25, wherein the amplitude of ionic flow blockage is measured.

27. The method of claim 1, further comprising providing a polymerase or exonuclease in one of the two pools, wherein the polymerase or exonuclease draws
15 the nucleic acid polymer through the channel.

28. The method of claim 27, wherein the medium is an aqueous solution.

29. The method of claim 28, wherein ionic flow between the two pools is
20 measured.

30. The method of claim 27, wherein ionic flow between the two pools is measured.

31. The method of claim 1, wherein the nucleic acid molecule contains modified adenosine and modified thymine which are not able to form base pairs, wherein the modified adenosine is capable of forming a base pair with unmodified thymine, and
5 wherein the modified thymine is capable of forming a base pair with unmodified adenosine.
32. The method of claim 1, wherein the nucleic acid molecule contains modified guanosine and modified cytosine which are not able to form base pairs, wherein the
10 modified guanosine is capable of forming a base pair with unmodified cytosine, and wherein the modified cytosine is capable of forming a base pair with unmodified guanosine .
33. The method of claim 1, wherein the nucleic acid molecule contains 2-
15 aminoadenosine, 2-thiothymidine, inosine, and pyrrolopyrimidine.
34. The method of claim 1, wherein the nucleic acid molecule contains 2-aminoadenosine, and 2-thiothymidine.
- 20 35. The method of claim 1, further comprising analyzing the nucleic acid molecules by electron tunneling.
36. A method of sequencing a nucleic acid molecule comprising steps of:

providing two separate, adjacent solutions of a medium and an interface between the two pools, the interface having a channel so dimensioned as to allow sequential nucleotide-by-nucleotide passage from one pool to the other pool of only one nucleic acid molecule at a time;

5 providing a nucleic acid molecule with at least one tandem repeat of a nucleotide sequence to be determined, wherein the nucleic acid molecule is synthesized using a circular template;

 placing the nucleic acid molecule in one of the two pools; and

 taking measurements as each of the nucleotides of the nucleic acid
10 molecule passes through the channel so as to determine the sequence of the nucleic acid molecule.

37. The method of claim 36, wherein the nucleic acid is single-stranded.

15 38. The method of claim 37, wherein the nucleic acid is single-stranded DNA.

39. The method of claim 37, wherein the nucleic acid is single-stranded RNA.

40. The method of claim 36, wherein the nucleic acid is an unstructured nucleic
20 acid.

41. The method of claim 36, wherein the circular template is single-stranded.

51. The method of claim 50, wherein the duration of ionic flow blockage is measured.

5 52. The method of claim 50, wherein the amplitude of ionic flow blockage is measured.

53. The method of claim 36, wherein the nucleic acid polymer interacts with an inner surface of the channel.

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54. The method of claim 53, wherein the medium is electrically conductive.

55. The method of claim 54, wherein the medium is an aqueous solution.

15 56. The method of claim 55, further comprising applying a voltage across the interface.

57. The method of claim 56, wherein ionic flow between the two pools is measured.

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58. The method of claim 57, further comprising applying a voltage across the interface.

59. The method of claim 58, wherein ionic flow between the two pools is measured.

60. The method of claim 59, wherein the duration of ionic flow blockage is
5 measured.

61. The method of claim 59, wherein the amplitude of ionic flow blockage is measured.

62. The method of claim 36, further comprising providing a polymerase or
10 exonuclease in one of the two pools, wherein the polymerase or exonuclease draws the nucleic acid polymer through the channel.

63. The method of claim 62, wherein the medium is an aqueous solution.
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64. The method of claim 63, wherein ionic flow between the two pools is measured.

65. The method of claim 62, wherein ionic flow between the two pools is
20 measured.

66. The method of claim 36, further comprising analyzing the nucleic acid by electron tunneling.

67. A method of sequencing a nucleic acid molecule comprising steps of:

providing two separate, adjacent solutions of a medium and an interface
between the two pools, the interface having a channel so dimensioned as to
allow sequential nucleotide-by-nucleotide passage from one pool to the other
pool of only one nucleic acid molecule at a time;

providing a nucleic acid molecule with modified nucleotides that reduce
secondary structure in the nucleic acid molecule;

placing the nucleic acid molecule in one of the two pools; and

taking measurements as each of the nucleotides of the nucleic acid
molecule passes through the channel so as to determine the sequence of the
nucleic acid molecule.

68. The method of claim 67, wherein the nucleic acid is single-stranded.

69. The method of claim 68, wherein the nucleic acid is single-stranded DNA.

70. The method of claim 68, wherein the nucleic acid is single-stranded RNA.

71. The method of claim 67, wherein the nucleic acid is an unstructured nucleic acid.

72. The method of claim 67, wherein the circular template is single-stranded.

73. The method of claim 67, wherein the circular template is double-stranded.

74. The method of claim 67, wherein the nucleic acid molecule contains modified
5 adenosine and modified thymine which are not able to form base pairs, wherein the
modified adenosine is capable of forming a base pair with unmodified thymine, and
wherein the modified thymine is capable of forming a base pair with unmodified
adenosine.

10 75. The method of claim 67, wherein the nucleic acid molecule contains modified
guanosine and modified cytosine which are not able to form base pairs, wherein the
modified guanosine is capable of forming a base pair with unmodified cytosine, and
wherein the modified cytosine is capable of forming a base pair with unmodified
guanosine .

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76. The method of claim 67, wherein the nucleic acid molecule contains 2-
aminoadenosine, 2-thiothymidine, inosine, and pyrrolopyrimidine.

77. The method of claim 67, wherein the nucleic acid molecule contains 2-
20 aminoadenosine, and 2-thiothymidine.

78. The method of claim 67, wherein the medium is electrically conductive.

79. The method of claim 78, wherein the medium is an aqueous solution.

80. The method of claim 79, further comprising applying a voltage across the interface.

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81. The method of claim 80, wherein ionic flow between the two pools is measured.

82. The method of claim 81, wherein the duration of ionic flow blockage is measured.

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83. The method of claim 81, wherein the amplitude of ionic flow blockage is measured.

84. The method of claim 78, further comprising applying a voltage across the interface.

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85. The method of claim 84, wherein ionic flow between the two pools is measured.

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86. The method of claim 85, wherein the duration of ionic flow blockage is measured.

87. The method of claim 84, wherein the amplitude of ionic flow blockage is measured.

88. The method of claim 67, wherein the nucleic acid polymer interacts with an
5 inner surface of the channel.

89. The method of claim 88, wherein the medium is electrically conductive.

90. The method of claim 89, wherein the medium is an aqueous solution.
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91. The method of claim 90, further comprising applying a voltage across the interface.

92. The method of claim 91, wherein ionic flow between the two pools is
15 measured.

93. The method of claim 92, further comprising applying a voltage across the interface.

20 94. The method of claim 93, wherein ionic flow between the two pools is measured.

95. The method of claim 94, wherein the duration of ionic flow blockage is measured.

96. The method of claim 94, wherein the amplitude of ionic flow blockage is
5 measured.

97. The method of claim 67, further comprising providing a polymerase or
exonuclease in one of the two pools, wherein the polymerase or exonuclease draws
the nucleic acid polymer through the channel.
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98. The method of claim 97, wherein the medium is an aqueous solution.

99. The method of claim 98, wherein ionic flow between the two pools is
measured.
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100. The method of claim 97, wherein ionic flow between the two pools is
measured.

101. The method of claim 67, further comprising analyzing the nucleic acid by
20 electron tunneling.

102. A method of producing a nucleic acid molecule with reduced secondary
structure, the method comprising steps of:

providing a circular nucleic acid template;

providing nucleotide precursors sufficient to synthesize the nucleic acid molecule using the nucleic acid template, wherein said precursors include pairs of complementary precursors, wherein the precursors in a complementary pair are characterized by a reduced ability to form base pairs with each other, and wherein at least one of the precursors in a pair is further characterized by an ability to form at least one base pair with another nucleotide;

providing an oligonucleotide primer capable of hybridizing to the template;

contacting the template, primer and the precursors with an enzyme characterized by an ability to polymerize the precursors under conditions and for a time sufficient for synthesis of the nucleic acid molecule containing multiple repeats of a sequence complementary to said template; and isolating said nucleic acid molecule.

103. The method of claim 102, wherein the nucleic acid is single-stranded DNA.

104. The method of claim 102, wherein the nucleic acid is single-stranded RNA.

105. The method of claim 102, wherein the nucleic acid is an unstructured nucleic acid.

106. The method of claim 102, wherein the circular template is single-stranded.

107. The method of claim 102, wherein the circular template is double-stranded.

108. The method of claim 102, wherein the precursors are selected from the group
5 consisting of: 2-aminoadensine triphosphate, 2-thiothymidine triphosphate, inosine triphosphate, and pyrrolopyrimidine triphosphate.

109. The method of claim 102, wherein the circular template is a single-stranded
template.

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110. A method of sequencing a double-stranded nucleic acid molecule comprising
steps of:

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providing two separate, adjacent solutions of a medium and an interface
between the two pools, the interface having a channel so dimensioned as to
allow sequential nucleotide-by-nucleotide passage from one pool to the other
pool of only one nucleic acid molecule at a time;

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providing a double-stranded nucleic acid molecule with at least one
repeat of a nucleotide sequence to be determined, wherein the nucleic acid
molecule is enzymatically synthesized using a circular template;
placing the double-stranded nucleic acid molecule in one of the two
pools; and

taking measurements as each of the nucleotides of the double-stranded nucleic acid molecule passes through the channel so as to determine the sequence of the nucleic acid molecule.

5 111. The method of claim 110, wherein the double-stranded nucleic acid is DNA.

112. The method of claim 110, wherein the double-stranded nucleic acid is RNA.

10 113. The method of claim 110, wherein the double-stranded nucleic acid is an unstructured nucleic acid.

114. The method of claim 110, wherein the circular template is single-stranded.

15 115. The method of claim 110, wherein the circular template is double stranded.

116. The method of claim 110, wherein the medium is electrically conductive.

117. The method of claim 116, wherein the medium is an aqueous solution.

20 118. The method of claim 117, further comprising applying a voltage across the interface.

119. The method of claim 118, wherein ionic flow between the two pools is measured.

120. The method of claim 119, wherein the duration of ionic flow blockage is
5 measured.

121. The method of claim 119, wherein the amplitude of ionic flow blockage is measured.

10 122. The method of claim 116, further comprising applying a voltage across the interface.

123. The method of claim 122, wherein ionic flow between the two pools is measured.

15 124. The method of claim 123, wherein the duration of ionic flow blockage is measured.

125. The method of claim 123, wherein the amplitude of ionic flow blockage is
20 measured.

126. The method of claim 110, wherein the nucleic acid polymer interacts with an inner surface of the channel.

127. The method of claim 126, wherein the medium is electrically conductive.

128. The method of claim 127, wherein the medium is an aqueous solution.

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129. The method of claim 128, further comprising applying a voltage across the interface.

10 130. The method of claim 129, wherein ionic flow between the two pools is measured.

131. The method of claim 130, further comprising applying a voltage across the interface.

15 132. The method of claim 131, wherein ionic flow between the two pools is measured.

133. The method of claim 132, wherein the duration of ionic flow blockage is measured.

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134. The method of claim 132, wherein the amplitude of ionic flow blockage is measured.

135. The method of claim 110, further comprising providing a polymerase or exonuclease in one of the two pools, wherein the polymerase or exonuclease draws the nucleic acid polymer through the channel.

5 136. The method of claim 135, wherein the medium is an aqueous solution.

137. The method of claim 136, wherein ionic flow between the two pools is measured.

10 138. The method of claim 135, wherein ionic flow between the two pools is measured.

139. The method of claim 110, wherein the nucleic acid molecule contains modified adenosine and modified thymine which are not able to form base pairs, wherein the
15 modified adenosine is capable of forming a base pair with unmodified thymine, and wherein the modified thymine is capable of forming a base pair with unmodified adenosine.

140. The method of claim 110, wherein the nucleic acid molecule contains modified
20 guanosine and modified cytosine which are not able to form base pairs, wherein the modified guanosine is capable of forming a base pair with unmodified cytosine, and wherein the modified cytosine is capable of forming a base pair with unmodified guanosine .

141. The method of claim 110, wherein the nucleic acid molecule contains 2-aminoadenosine, 2-thiothymidine, inosine, and pyrrolopyrimidine.

5 142. The method of claim 110, wherein the nucleic acid molecule contains 2-aminoadenosine, and 2-thiothymidine.

143. The method of claim 110, further comprising analyzing the nucleic acid molecules by electron tunneling.

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